

Deficiency and Excess of Essential and Non-essential Metals in Terrestrial Insects

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Abstract

Essential elements such as iron, copper and zinc form integral parts of cellular processes and are required in trace amounts by insects if they are to grow and reproduce normally. The ability to regulate assimilation and excretion of these elements via the digestive tract was an essential prerequisite for their successful colonization of the land. However, when present at high concentrations due to pollution, these essential metals (and those that are not required such as cadmium, lead and mercury) may disrupt normal biochemistry. Sensitive insects may be killed by acute or chronic poisoning, or die from deficiency of an essential element through antagonism from a non-essential metal in the diet. Species that are tolerant to pollution may respond to the subsequent lack of competition and reach much higher population densities than in uncontaminated

areas. These changes at the community level may lead to disruption of ecological processes such as plant litter decomposition.

Examples of these phenomena are described in terrestrial insects. Special attention is paid to Collembola and Diptera in which rapid evolution of resistance occurs in response to metal contamination.

I. INTRODUCTION

Metals are natural substances. With the exception of radioisotopes produced in nuclear reactors, all metals have been an integral part of the biosphere throughout evolution. Elements that are considered to be metals for the purpose of this review are shown in Fig. 1.

Metals are also non-biodegradable. Unlike many organic pesticides and pollutants, they cannot be broken down into less-harmful components. Detoxification consists of "hiding" active metal ions within a protein, or depositing them in an insoluble form in intracellular granules for long-term storage or excretion in the faeces.

A further important feature of some metals is their essentiality (Fig. 2). Metals such as copper and zinc have a window of essentiality within which

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Rf	Ha													
		Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		
		Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr		

Fig. 1. Periodic table of the elements. Those considered to be metals are surrounded by bold lines. Metalloids (with properties of metals and non-metals) are shaded. From Hopkin (1989), by permission of Elsevier Applied Science.

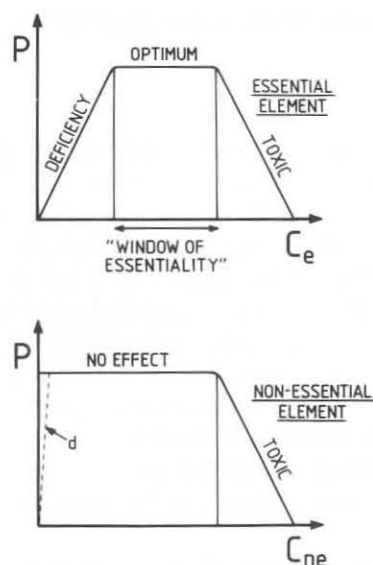


Fig. 2. Relationships between performance (P) (growth, fecundity, survival) and concentrations of an essential (C_e) or non-essential element (C_{ne}) in the diet of animals. Possible deficiency effects at ultra-trace levels (d) of an apparently non-essential element may be discovered as the sensitivities of analytical techniques are improved. From Hopkin (1989), by permission of Elsevier Applied Science.

dietary concentrations of the elements must be maintained. However, in terms of availability of these essential metals (and non-essential elements such as cadmium, mercury and lead), insects living in industrialized regions are in an environment that is much-changed from that in which regulatory processes for metals evolved.

In this review, the roles that metals play in the biology of terrestrial insects will be discussed. Most citations are to work published since the review of Hopkin (1989) which contains a comprehensive list of earlier literature. After considering essentiality (Section II), the responses of insects to metal pollution are examined (Section III). It is clear that adaptation to increased availability of metals involves the enhancement of existing regulatory and detoxification mechanisms, rather than the development of new systems. This has interesting implications for insect ecology and pest control which are discussed in the final part (Section IV).

II. NATURAL SELECTION OF THE ELEMENTS

A. Introduction

Life evolved in the sea some 3.5×10^9 years ago. The solubility of elements under the anaerobic conditions that existed at the time determined which metals could be incorporated into biochemical reactions. A natural selection of the elements took place where the choice of one element rather than a similar one was dictated by its availability, the ability of the organism to retain it, and its functional advantages relative to those of other metals (Williams, 1981). When the atmosphere changed from being chemically reducing, to one containing 20% oxygen, organisms had to develop aerobic biochemistry. One of the greatest changes was in the availability of iron which became almost completely insoluble. Thus proteins for storage (ferritin) and transport (transferrin) of this metal had to be developed (Locke and Leung, 1984; Locke and Nichol, 1992).

The earliest known hexapod is a collembolan *Rhyniella praecursor* Hirst & Maulik from the Rhynie chert formed in the Lower Devonian (Greenslade and Whalley, 1986). The earliest insect communities probably developed under algal mats and later, on low emergent vegetation (Little, 1990; Shear and Kukalová-Peck, 1990; Kukalová-Peck, 1991, 1992; Edwards and Selden, 1992). It is likely that the food of these early insects consisted of fungal hyphae, which were grazed directly, and bacteria that were stripped from fragments of decaying vegetation as they passed through the gut (Price, 1988).

Concentrations of metals in fungi are usually much higher than the substrate on which they are growing (Starling and Ross, 1991; Hopkin, 1993a). Thus from the time of the emergence on to land of the first insects, systems for metal regulation would have to have been well developed. The option of excreting unwanted elements directly to the surrounding water was no longer available. The ability to regulate assimilation and excretion of essential and non-essential metals via the digestive tract was an essential prerequisite for the successful colonization of the land by insects.

B. Deficiency of Essential Metals

Concentrations of some essential elements in the diets of terrestrial insects are quite low in comparison to minimum dietary requirements (Roth, 1992; Studier and Sevick, 1992). For example, the level of copper

in the leaves of deciduous trees is often less than $10 \mu\text{g g}^{-1}$ dry weight (Guha and Mitchell, 1966; Flemming and Trevors, 1989; Markert, 1992). In some parts of the world, levels of copper (and other essential elements) in soils are so low that growth of plants is restricted (Boardman and McGuire, 1990a,b; Hunter *et al.*, 1990). Deficiency diseases in livestock are well documented in such areas, but little attention has been paid to potential effects on invertebrates. Is there any evidence for metal deficiency in insects?

Creighton (1938) was the first author to examine this question. He reported very high mortality of lepidopteran larvae fed on foliage from copper- and zinc-deficient plants. There is clear experimental evidence for a minimum dietary requirement for copper, iron, manganese and zinc in aphids (Fig. 3; Dadd, 1967; Akey and Beck, 1972), copper and zinc in crickets (McFarlane, 1976), and zinc in beetles (Fig. 4; Fraenkel, 1959). Some insects go to great lengths to assimilate essential elements and to retain them once they are in the body. Bruchid beetles, for example, assimilate up to 90% of the copper and zinc from the seeds on which they feed (Ernst, 1992, 1993). The male of the noctuid moth *Heliothis virescens* (Fabricius) transfers 36% of its whole body zinc content to the female at the time of mating (Engelbreton and Mason, 1980).

Thus, metal deficiency has been demonstrated in several insect groups in the laboratory and some species have evolved to maximize the assimilation and retention of essential elements. However, direct evidence is lacking of the possible effects of these phenomena on insect populations. Dietary switching in grasshoppers (Bernays and Bright, 1991) and other insects (Waldbauer and Friedman, 1991) may occur in response to nutrient deficiency, but there is as yet no evidence that such behaviour could be stimulated by inadequate levels of an essential metal in the food. This is clearly an area in need of more research.

C. Regulation of Metals

The pathways of metal detoxification in invertebrates are relatively well understood (for reviews see Hopkin, 1989, 1990; Hopkin *et al.*, 1989; Beeby, 1991; Hare, 1992; Dallinger, 1993; Heliövaara and Väisänen, 1993). The epithelium of the midgut acts as a barrier separating the contents of the lumen from the haemolymph. Thus the midgut usually contains the highest concentrations of metals in insects (Fig. 5; Table 1; Lauerjat *et al.*, 1989; Raes *et al.*, 1992). A pool of available essential metals is maintained in the cells to supply the needs of normal biochemistry (e.g. 40% of total copper in the midgut of *Lucilia cuprina*

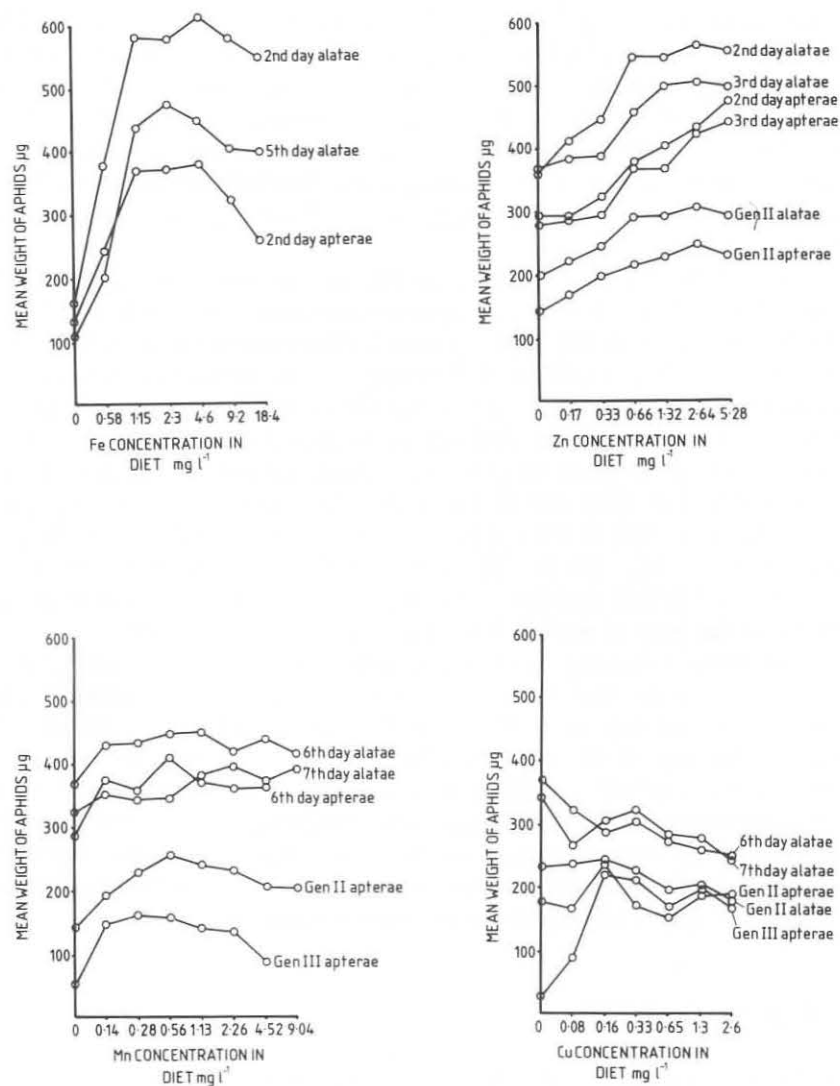


Fig. 3. Mean individual weights of the aphid *Myzus persicae* (Sulzer) after 8 days' growth on a synthetic diet containing a range of concentrations of iron, zinc, manganese and copper. 2nd, 3rd, 6th and 7th day apterae and alatae are larvae of the first generation on a synthetic diet weighed on the 8th day of growth. Gen II and Gen III are larvae of the second and third generations weighed when apterae had become adults on the best diets. Note the classic "bell-shaped" dose-response curve for iron. Redrawn from Dadd (1967) by permission of Pergamon Journals.

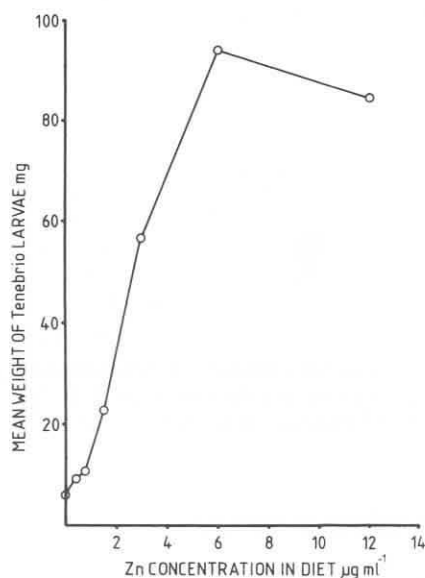


Fig. 4. Graph showing the mean individual weight of larvae of *Tenebrio molitor* L. reared for 11 weeks on a synthetic diet containing different levels of zinc ($n = 20$ for each treatment). The beetles require a concentration of zinc in their food of at least $6 \mu\text{g g}^{-1}$ (fresh weight) for normal growth. Based on data in Fraenkel (1959).

(Wiedemann) is water soluble – Waterhouse, 1945). Essential metals that are surplus to requirements, and non-essential metals, are prevented from reaching the other body tissues by storage in proteins and insoluble granules.

There are three main pathways of intracellular metal detoxification in insects (Hopkin, 1989). Metals following the *Type A pathway*, such as calcium, zinc and manganese, are stored as phosphates in concentrically structured granules (Ballan-Dufrançais *et al.*, 1980; Jeantet *et al.*, 1980). Metals following the *Type B pathway*, such as cadmium, mercury and copper, are bound initially in metallothionein proteins (Aoki *et al.*, 1984; Suzuki *et al.*, 1989; Cosson *et al.*, 1991; Theodore *et al.*, 1991), and are deposited eventually in specialized lysosomes as dense, sulphur-rich, insoluble bodies (Ballan-Dufrançais *et al.*, 1980; Jeantet *et al.*, 1980). The *Type C pathway* is followed by iron (Locke and Leung, 1984; Locke and Nichol, 1992). Iron is stored in ferritin. However, surplus iron may be deposited in modified lysosomes in a similar way to metals that follow the type B pathway. All metals stored in granules may be excreted in the faeces following lysis of the midgut cells.

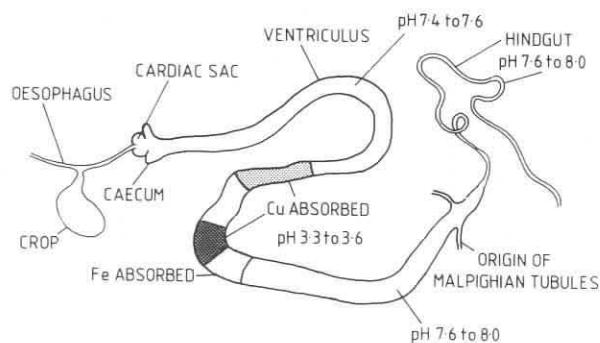


Fig. 5. Diagram of the gut of *Lucilia cuprina* larva (Diptera) showing pH of gut contents and regions of iron and copper absorption. Redrawn from R. F. Chapman, English Universities Press, London, "The Insects: Structure and Function", 1971, by permission of Hodder & Stoughton.

D. Critical Concentrations of Metals

Attempts have been made to set critical concentrations for metals in terrestrial ecosystems that will protect most species from the effects of pollution (Bengtsson and Tranvik, 1989; Van Straalen, 1993). However, this is extremely difficult to apply to insects owing to their diversity of feeding behaviours and habitats. Indeed, Hopkin (1993b) has speculated that under some circumstances, a critical concentration in soil that protects one species from poisoning may be below the minimum dietary requirement for another.

Table 1. Distribution of cadmium in larvae of the fleshfly *Sarcophaga peregrina* after feeding for 3 days on homogenized porcine liver containing $100 \mu\text{g Cd g}^{-1}$ as CdCl_2 . From Aoki *et al.* (1984) by permission of Pergamon Press.

	Content ($\mu\text{g/larva}$)	Distribution (%)	Concentration ($\mu\text{g g}^{-1}$ wet tissue)
Whole body	$1.53 \pm 0.19^*$	100	$14.6 \pm 2.2^*$
Digestive tract	$1.37 \pm 0.49^*$	90	$301 \pm 81^*$
Fat body	0.009^\dagger	0.6	0.1^\dagger
Malpighian tube	0.036^\dagger	2.3	9.0^\dagger
Trachea	ND		
Brain	ND		
Cuticle	0.062^\dagger	4.1	0.34^\dagger

*Means \pm SD ($N = 5$).

† Mean value of pooled samples.

ND: Not detectable.

In the future, it is important that the windows of essentiality for metals such as copper and zinc are considered in the context of the biology of the animals one is attempting to protect. In the same way as the choice of a management regime on a nature reserve dictates which insect species will thrive and which will perish, we may have to choose a critical concentration that will benefit those species we wish to protect at the expense of others. At present we are nowhere near being able to make such a decision owing to the lack of information on the potential effects of metal deficiency on insects.

III. METAL POLLUTION

A. Introduction

Present-day insects in industrialized countries are living in a changed environment in comparison to that in which they evolved. Several metals are present in much greater concentrations in soils and vegetation than before the industrial revolution. For many metals such as cadmium, lead and mercury, the activities of Man contribute a much greater proportion of total global release to the atmosphere than emissions from volcanoes and other natural phenomena (Nriagu, 1988, 1990; Nriagu and Pacyna, 1988).

One of the largely unexplored side-effects of the use of metal-containing insecticides is their possible role in rectifying essential element deficiencies. Creighton (1938) remarked on the fact that populations of some insects show a temporary increase following application of Bordeaux mixture, a copper-containing pesticide that was used extensively before the development of organic insecticides. However, the greatest impact of metals on insect populations has undoubtedly been in response to excess from pollution rather than deficiency. The effects of metal pollution can be categorized into four main areas, community effects (Section IIIB), bioaccumulation (Section IIIC), effects on individuals and species (Section IIID), and genetics (Section IIIE).

B. Community Effects

When studying effects of metal pollution on communities, polluted sites are usually compared with an uncontaminated reference site which has similar characteristics (soil type, climate, vegetation etc.). However, unless the polluted site is heavily contaminated, it is difficult to attribute

differences in insect communities to metal pollution rather than natural fluctuations.

One approach that has been developed extensively for freshwaters (Wright *et al.*, 1989), but has received relatively little attention in insects (Stork and Eggleton, 1992), is to predict the probabilities of species occurring in a site and then to compare the predictions with the actual species present. In contaminated sites, it may be possible to attribute absences to metal pollution. However, such an approach relies on a very detailed knowledge of the fauna of many habitat types which is not presently available for most groups.

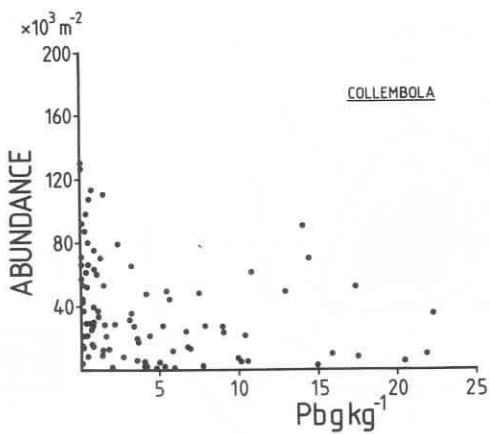
Despite these limitations, clear evidence of effects of metal pollution on insect communities has been obtained. Collembola are particularly susceptible and there are numerous examples of substantial changes in relative abundances of species near to sources of metal pollution (Fig. 6; Bengtsson *et al.*, 1985; Bengtsson and Rundgren, 1988; Tranvik and Eijsackers, 1989; Tranvik *et al.*, 1993).

C. Bioaccumulation

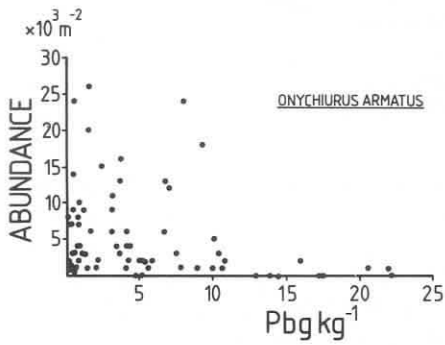
Insects which have evolved to deal with a wide range of metal concentrations in their diet (e.g. non-specialized carnivores with a large detoxification safety margin) are more likely to survive metal contamination of their food than those in which the levels are highly predictable and within narrow limits (e.g. sap-suckers with a narrow detoxification safety margin). For example, the pine bark bug *Aradus cinnamomeus* Panzer has a discontinuous gut. Under normal circumstances, levels of metals in the sap on which it feeds are so low that there is sufficient detoxification capacity for it to store all the metals ingested. However, this renders the species vulnerable to poisoning if its diet is contaminated. In Finland, *A. cinnamomeus* is absent from the close vicinity of a factory that emits substantial amounts of metal pollution (Heliövaara *et al.*, 1987). Specimens collected from trees further away contain elevated concentrations of a range of metals including copper (Fig. 7).

Fig. 6. Abundance of (A) total Collembola, (B) *Onychiurus armatus* (Tullberg) (Collembola) and (C) *Isotoma olivacea* Tullberg (Collembola) in the 0–3 cm layer in lead-contaminated soils in the vicinity of a natural metalliferous outcrop in a Norwegian spruce forest. The concentrations of lead represent metal extracted from soil over 18 h in 0.1 M buffered acetic acid. Note that *O. armatus* is sensitive to lead pollution whereas *I. olivacea* reaches higher population densities in contaminated soils. Redrawn from Hågvar and Abrahamsen (1990) by permission of the Entomological Society of America.

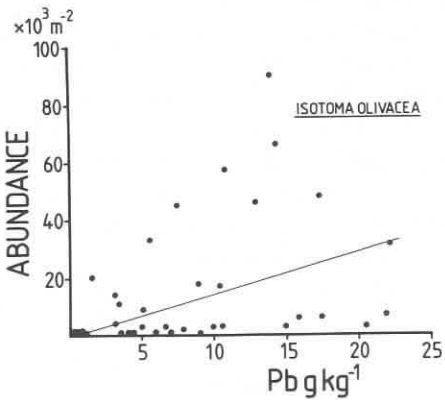
(A)



(B)



(C)



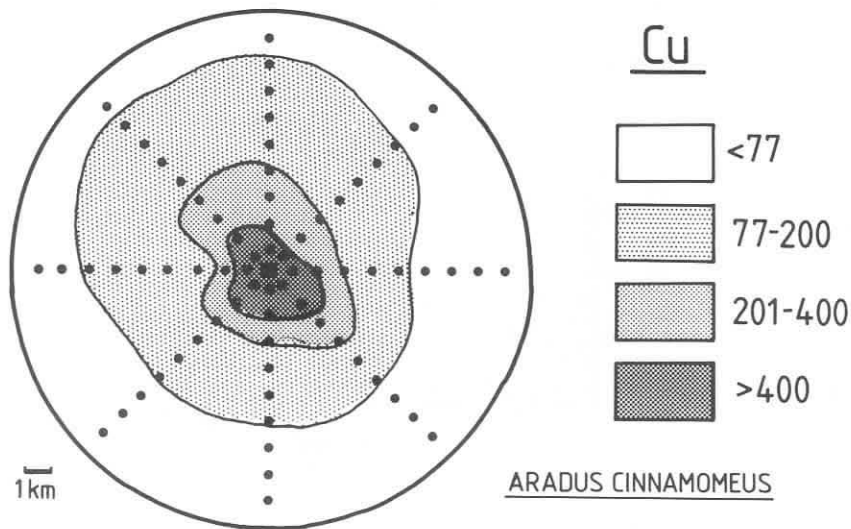


Fig. 7. Mean concentrations of copper ($\mu\text{g g}^{-1}$) expressed as isolines in pine bark bugs *Aradus cinnamomeus* around a factory (■) in Finland. Lines of dots indicate eight 9-km radial transects. Redrawn from Heliövaara and Väisänen (1987) by permission of Elsevier Applied Science.

Any metals that are in the body of an insect may be transferred to the next level in the food chain if that insect is eaten by a predator. However, it is important to recognize that there are large differences in the concentrations of metals between species in the same habitat (Heliövaara and Väisänen, 1989, 1990, 1991; Lindqvist, 1992; Janssen and Hogervorst, 1993). Indeed, it is impossible to generalize about the role of insects in food chain transfer of metals. A more useful concept is that of "critical pathways" where knowledge of the feeding biology and detoxification mechanisms enables one to predict which species will be most at risk from metal pollution (Beyer, 1986; Fagerström, 1991; Laskowski, 1991; Van Straalen and Ernst, 1991; Streit, 1992). Thus around the factory site in Finland described above, the concentrations of cadmium, copper, nickel and lead in larvae of the pine resin gall moth *Petrova resinella* (L.) were only about one tenth of those in *A. cinnamomeus*. Predators of *P. resinella* are clearly less at risk from metal poisoning than those that eat *A. cinnamomeus*.

D. Effects on Individuals and Species

Assimilation of non-essential metals above a critical concentration, and essential metals above the window of essentiality, may lead to decreased

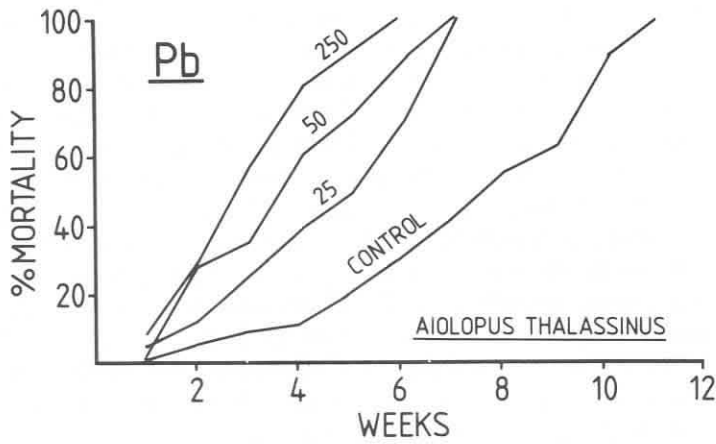


Fig. 8. Post-eclosion mortality of F1 adults of the grasshopper *Aiolopus thalassinus* (Fabricius) reared from eggs laid in soil treated with lead. The figures by each line represent the concentration of lead ($\mu\text{g g}^{-1}$ dry weight) in the experimental soils. Redrawn from Schmidt *et al.* (1991) by permission of Elsevier Science Publishers.

growth and reproduction, and increased mortality (Fig. 8). If a sufficient proportion of a population is affected in the field, then the species will become extinct unless it can evolve resistance (see Section IIIE).

Some effects may be more subtle than simple poisoning. For example, detoxification of metals by insects in polluted sites involves expenditure of energy that could otherwise be used for purposes such as growth and reproduction. This may lead to longer development times (Creighton, 1938; Cohn *et al.*, 1992). A general weakening of individual insects within a species may make them more susceptible to parasites (Ortel *et al.*, 1993).

Effects may be more subtle than simple presence or absence. For example, Read *et al.* (1987) showed that the carabid beetle *Nebria brevicollis* (Fabricius) did not exhibit a summer diapause in a metal-contaminated woodland near to a primary cadmium, lead and zinc smelting works. This may have been due to the scarcity of prey that had been killed by metal pollution. A species of beetle discovered recently in Finland prospers in pollution-damaged conifers (Heliövaara *et al.*, 1990).

E. Genetic Effects

Some species of insects are able to *tolerate* higher environmental concentrations of metal pollutants than others because they are pre-adapted to cope with a wide range of metal concentrations in their food

(Section IIIC). However, for a population to be considered to be *resistant*, it must be genetically different from a non-resistant population.

At present, there is clear experimental evidence for the evolution of metal-resistant races of *Drosophila* (Maroni *et al.*, 1987; Theodore *et al.*, 1991) and Collembola (Posthuma, 1990; Frati *et al.*, 1992; Posthuma *et al.*, 1992, 1993a,b), although the phenomenon is certain to be detected in other groups of insects in the future. The topic has recently been reviewed comprehensively by Posthuma and Van Straalen (1993).

In Collembola, the basis of resistance to metal pollution is an increased ability to excrete assimilated metal (Posthuma *et al.*, 1992). Metals are detoxified in insoluble intracellular granules in the midgut (Van Straalen *et al.*, 1987). During moulting in Collembola, the epithelium of the midgut is shed along with the exoskeleton. Hence, an increased capacity for metal storage in granules leads to a greater excretion efficiency. In resistant populations, this adaptation is passed on to the next generation (Posthuma, 1990).

One way of increasing tolerance to pollutants is to increase the copy number or the transcription rate of the gene coding for detoxifying proteins. In the case of organophosphate insecticides, up to a 256-fold amplification of the genes coding for non-specific esterases that break down the insecticide have been found in mosquitoes (Devonshire and Field, 1991). For metals such as copper and cadmium in *Drosophila*, the genes that code for the metal-binding protein metallothionein may be duplicated up to four times. The metals are bound more rapidly in resistant animals following ingestion (Maroni *et al.*, 1987; Theodore *et al.*, 1991). Such amplification has been found in wild *Drosophila* populations and has probably evolved in response to the spraying of fruit trees with copper-containing fungicides. This provides a good example of how one species of insect has responded at the biochemical level to a changed environment.

IV. CONCLUSIONS

Metals have been present throughout the evolution of terrestrial insects. Different species have evolved a range of strategies to regulate internal concentrations of essential and non-essential metals that they ingest with their food. Since the industrial revolution, the availability of many of these elements has increased owing to pollution. Some species are able to survive because they are pre-adapted to cope with a wide range of metal concentrations in their diet (e.g. polyphages). Others are vulnerable to poisoning since the levels of metals in their food are normally within

narrow limits (e.g. sap suckers). Some (e.g. *Drosophila*) have been able to evolve resistance to their changed environment by enhancing existing detoxification mechanisms.

Insects provide a route for transport of metals to higher levels in food chains. Indeed, some species may be suitable as biological monitors of pollution. (Samiullah, 1990; Hopkin, 1993c). However, it is important to recognize that the extent of metal accumulation is species-specific. Critical pathways of metal transfer should be identified based on basic biological knowledge of feeding behaviour, life histories and other ecological factors.

One largely unexplored area is the possible co-evolution of phytophagous insects and their food plants in terms of metal availability. One response of a plant to insect attack might be to restrict the availability of an essential metal such as copper, thus reducing the insects' performance. This possibility has been discussed in the literature with regard to major nutrients (Haukioja *et al.*, 1991; Lindroth *et al.*, 1991). However, I am not aware of essential metals being considered in this context. As Lawton (1986) remarked, "All three groups working on birch are agreed that other, as yet unknown, chemical changes probably take place in damaged leaves, and could be much more important for herbivores than crude observations on increases in total phenolics". Restriction of essential trace element availability by plants could be one such chemical change.

The opposite response of plants to insect attack would be to hyperaccumulate metals until their concentrations in the leaves reached toxic levels. Hyperaccumulation is well known in metallophytes (Baker and Proctor, 1990; Ernst *et al.*, 1992). In Central Queensland for example, leaves of *Stackhousia tryonii* Bailey may contain up to 2% nickel on a dry weight basis (Batianoff *et al.*, 1990). Do these plants accumulate such high levels of metals as a feeding deterrent?

The whole question of whether insects ever suffer from essential metal deficiency in the wild is almost completely unexplored. Many species show a slight increase in performance in response to low levels of contaminants in their diets. This is usually attributed to hormesis, the stimulation of growth by low levels of inhibitors (Stebbing, 1982; Van Ewick and Hoekstra, 1993). However, it is feasible that a slight supplementation of the diet of an insect, with say copper, could rectify a deficiency and improve performance.

If this conjecture is true, then new approaches to insect control may be possible. For example, varieties of crops could be selected to have a concentration of an essential metal that was below the window of essentiality for an insect pest, thus restricting attack. Pesticides could be developed that interfered with the biochemical regulation of essential

metals in insects. In theory, the insect could not evolve resistance to a deficiency as there are no substitutes for the biochemical roles of essential elements.

It would seem reasonable to press ahead with attempts to reduce the emissions of metals to the environment. Metals are persistent pollutants and have extremely long residence times in soils. The continuous build-up of concentrations may have long-term consequences for insect populations which we are only just beginning to understand.

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